Changes in Echolocation Calls of *Eptesicus fuscus* When Flying with Conspecifics in a Laboratory Setting

Bethany M. King

Introduction

Echolocation calls have long been studied in bats not only because of their navigational properties, but also for their potential communicating properties. Many studies have shown that echolocation calls can influence the behavior of conspecifics, meaning that there is a definite social aspect to these calls. They may convey information such as group identity (Kazial, Burnett, and Masters, 2001; Boughman and Wilkinson, 1998) and sex (Kazial and Masters, 2004). Variation in the echolocation calls has been found related to other factors as well, as explained in the next paragraph, and other bats may be able to detect on this variation. In fact, echolocation calls may provide nearly as much information to other bats as social calls do (Fenton, 2003).

Variation in bat echolocation calls has been studied under many conditions, and has been found to be correlated with many factors, for instance, prey type (Leippert et al. 2002), habitat (Obrist, 1995), individual identity (Burnett, Kazial, and Masters, 2001), and presence of conspecifics (Obrist, 1995; Ratcliffe et al., 2004). Different aspects of the call may be changed, and often a change in one variable is correlated with a change in other variables (Jones, 1999). Some of the most variable aspects are call duration, time between successive calls, frequency range utilized, the sweep of frequency over time, overall amplitude, and the frequency and time of the maximum amplitude of a call (Surlykke and Moss, 2000; Masters, Jacobs, and Simmons, 1991; Kazial, Burnett, and Masters, 2001).

Most of the studies mentioned have focused on recording bats in natural conditions, which presents the potential problem of variation in calls based on environmental cues rather than strictly individual or group variation (Leippert et al. 2002; Obrist, 1995; Ratcliffe et al., 2004; Boughman, and Wilkinson, 1998; etc.). The present study attempted to minimize environmental differences and distractions by flying and recording the bats in a standardized laboratory setting. The emitted echolocation calls could then all be recorded in the same way. Some studies have suggested that bats do not use echolocation calls in the laboratory identical to those in the wild (Surlykke and Moss, 2000). However, this is most likely to be a response to the smaller space. This difference should not be a problem in the present experiment because the goals of this experiment are to compare the calls of a bat flying alone in the laboratory to the calls of that bat flying with another bat in the laboratory. Any changes we find related to the presence of conspecifics should be applicable to bats in the wild as well, because we would expect similar changes over a longer, slower time scale. We hypothesized that there would be a change in the calls to avoid mutual interference (jamming), and thought that these changes would occur in consistent ways. The goal was to find a variable or collection of variables that the bat could potentially control that changed in a consistent manner between calls alone and calls with another bat.

Methods

I used six bats previously captured from around the Columbus area between February 2002 and October 2003. Two were captured at Whetstone Park in Clintonville, one at the Park of Roses in Clintonville, two on the Ohio State University campus, and one in downtown Columbus. All bats had been in the laboratory for over a year at the time of the experiment, so they were considered to be adults. The bats were housed in individual cages and kept on a 12/12 hour reversed light/dark schedule. They were fed mealworms (*Tenebrio molitor* larvae) which had been raised on flour supplemented with vitamins and minerals as suggested by Barnard (1985). They were also provided with vitamin and mineral supplemented water *ad libitum*.

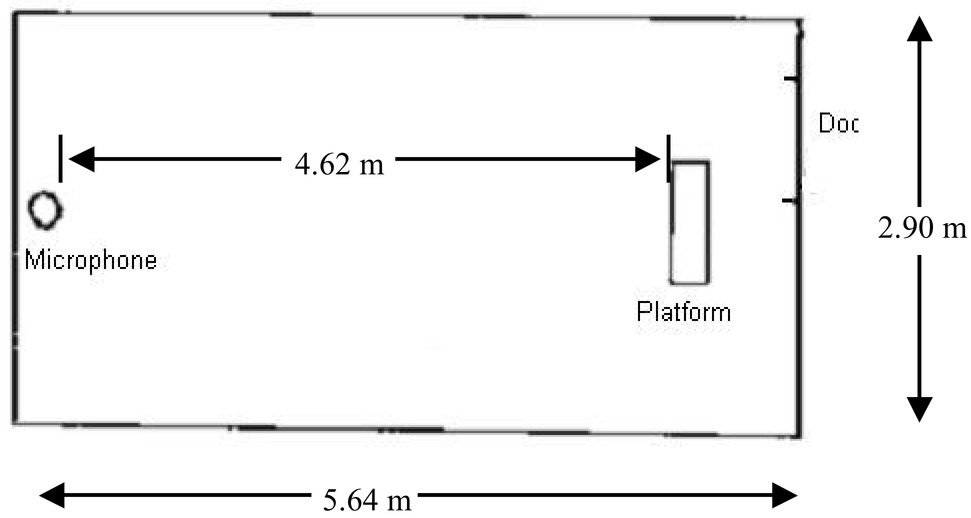


Figure 1: The setup of the recording room, which measured 5.64m X 2.90m X 2.75m

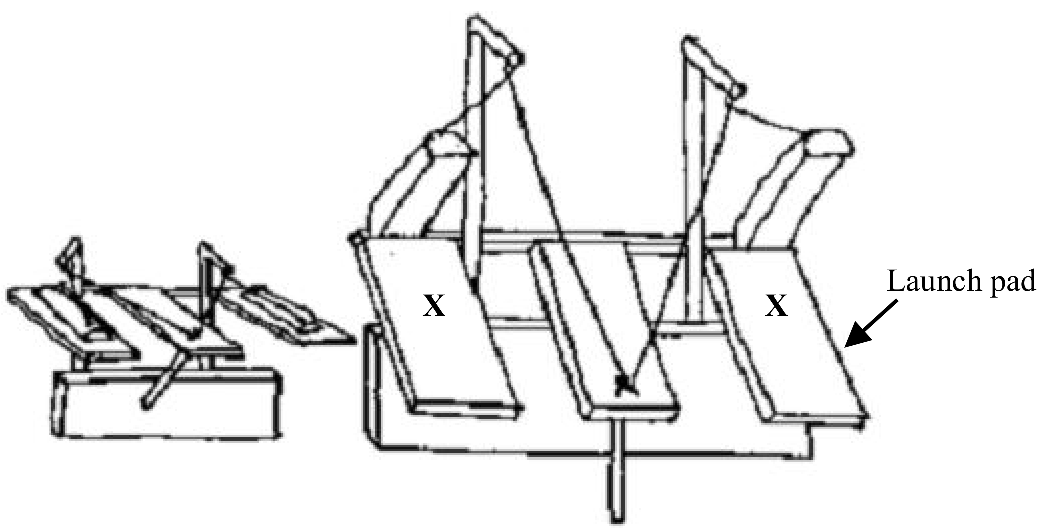


Figure 2: The launching platform in the closed (left) and open (right) configurations. **X** denotes where the bat sits. The platform was 1.37m high at the level of the bats.

The bats were acclimated to flying in a room that was lined with sound absorbent foam on the walls and ceiling, and several layers of carpeting on the floor, making it relatively anechoic. A release platform was located at one end of the room and the recording microphone was centered at the other end (Fig. 1). The bats were trained to fly to the other end of the room when launched from the platform either singly or together. The launch platform (Fig. 2) was made of wood and allowed two Plexiglas launch "pads" to be tilted simultaneously. Each launch pad was covered by a plastic dome to hold the bat in prior to release. When the launch pads were tilted and the domes raised, that signaled the bats to take off and begin flying.

Two people were used to record the bat calls, one operated the computer and the other handled the bats. Calls were recorded directly to computer disk using NIDisk (Engineering Design, 2002) at a sample rate of 454 kHz. Recording time was set at 3 seconds for each trial, giving ample time for the bat to fly to the other end of the room. The computer was a Toshiba laptop running on AC power, which was kept outside the experimental room because the laptop screen gave off an ultrasonic sound. To ensure that the recording equipment was started before or just at the time that the bats were released, we used a remote control switch to start recording that could be operated from inside the experiment room.

A	B	Date
Winston	Brent	April 25
Farah	Dedalus	April 25
Rose	Lenny	April 27
Brent	Dedalus	May 2
Farah	Rose	May 2
Winston	Lenny	May 4
Brent	Farah	May 4
Dedalus	Rose	May 9
Winston	Farah	May 11
Brent	Lenny	May 11
Lenny	Farah	May 16
Dedalus	Winston	May 16
Rose	Brent	May 18
Lenny	Dedalus	May 18
Rose	Winston	May 23

Table 1: Designation of "bat A" and "bat B" on a pair by pair basis. Designations were made randomly each day for each pair, so that no bat always went first, or always went second.

Each bat was flown in a pair once with each other bat, resulting in 15 pairs. No bat was tested more than once in two days (Table 1). On each day, a pair of bats were flown in a pattern of: A, B, A&B, B, A, B&A. Two flights were recorded for each turn (i.e. bat A flew twice, bat B flew twice, then they flew twice together). When not being flown, bats were kept out of the experiment room. We varied the starting side of the release platform such that bat A start on the right side and bat B on the left the first half, and bat B on the right side with bat A on the left for the second half. One or two pairs of bats were recorded in a day, so each individual was recorded over several weeks. Of the bats used, Rose was the only female.

Calls were automatically extracted, and analyzed using a specially written MATLAB program (The MathWorks, 2005). When two bats were together, the program automatically extracted calls, but I had to look at each call to ensure that it was not two overlapping calls. After extraction to individual files, the calls were analyzed with a different specific program in MATLAB. This program determined call length by first finding the maximum amplitude, and then scanning in either direction for when the amplitude had dropped by 20 db. This worked well for five of the bats, but the sixth bat, Rose, had calls that were generally softer than the others, so her call lengths were based on a 17 db drop. For recordings in which Rose was flying with another bat, calls were extracted using both thresholds. For calls assigned to Rose the 17 db threshold was used and for calls assigned to the other bats the 20 db threshold was used. The analysis program extracted the value of 40 parameters for each call it found, based on frequency and time measurements, to describe the course of the fundamental frequency, and tried to match the call to one of 6 possible models to describe its course. It rejected any call where parameters could not be assigned values, or that failed to fit any of the 6 possible models. The extracted values were transferred to a statistical program, SPSS 13.0 (SPSS Inc., 2004), for further analysis.

Statistical Methods and Results

The first problem was to determine which calls went with which bat in the paired situations. I used a discriminant function analysis (DFA) to assign calls to bats. To do this, all calls involved in a particular pair's recording were combined into a single file and I performed a principle components analysis (PCA) on calls each bat made when flying alone, keeping only those components whose eigenvalue was ≥ 1.0 (Lachenbruch, 1968). PCA was necessary to avoid the possibility of segregating calls based on fortuitous match up of variables with irrelevant background noise, which has been shown to be especially problematic when the ratio of variables to observations is high. Performing PCA reduced the 40 variables to 6 or 7, depending on the pair. The principle components were then used to perform the DFA and assign each call to either bat A or bat B. To assign calls for the next step, I used only calls for a particular bat that the DFA had assigned to that bat with a 95% or better certainty.

The next question was whether a DFA could identify calls from an individual bat as being emitted when that bat was alone vs. with another bat. To do this I reorganized the data so that all the calls of each bat were in one file. I performed a new PCA of these calls and used those results to perform a series of DFAs. My hypothesis was that the significance levels for a DFA of a bat flying alone vs. together (AvT) would be lower (i.e. more significant) than the level of either alone vs. alone (AvA) or together vs. together (TvT) comparisons. Alone means that the bat was flying alone at the time and together means that it was flying with another bat. I expected that the bat would change his or her calls when flying with another bat, and therefore there would be a greater difference between a sample of calls from the bat flying alone and a sample from the bat flying together than the difference within either one of those groups. A new PCA was performed for each bat, which reduced the variables from 40 to 7 or 8, depending on the individual. These variables were used in several different DFAs described below.

Before proceeding with the tests of alone vs. together comparisons, it was important to know if a bat changed his or her alone call between the first two flights alone (before having flown with another bat) and the second two (after having flown with another bat). To test this, I sorted the calls into groups of together, alone before flying with another bat, and alone after having flown with another bat, and performed a DFA on the alone groups. Despite a fairly high degree of misclassification, the results were significant (Table 2). This showed that in order to combine the alone calls for analysis against the together calls, I was going to have to be very careful to use a random sample to avoid biasing the results. There are several possibilities for the high significance of this result. One is obviously that the bat had flown with another bat and perhaps maintained any change it made to accommodate the other bat's presence. Another possibility is simply that the bat had had more time to warm up, between the first period and the second. Further research would be necessary to find out why there was such a high degree of significance in this test.

Bat	**Significance Level**	**Percent Misclassified**
Brent	0.000*	21.0%
Dedalus	0.000*	17.4
Farah	0.000*	13.3
Lenny	0.013*	23.0
Rose	0.059	16.4
Winston	0.000*	18.5

Table 2: Significance and misclassification values for bat calls divided into alone before flying with another bat and alone after flying with another bat. * denotes significance $P \leq 0.05$

To actually test the ability to discriminate between calls of a bat alone and a bat with another bat, I took a random sample of 60 calls from when the bat was flying alone, and assigned odd ones to group 1 and even ones to group 2, giving me a random half in each group. I then performed a DFA to get a significance value of discriminability between the two random groups of AvA. The same process was repeated using only calls from the bat when it was recorded with another bat for a TvT comparison. I expected that since the sample was random, the DFA should not work well to discriminate between the two groups in either test. Lastly, 30 calls were drawn at random form those emitted when the bat was flying alone and 30 from the bat flying together. DFA was used to see if they could be separated in an AvT comparison. This whole process was then repeated for each bat.

Results of the DFA are shown in table 3. The significance was in fact lower for the AvT group than for either of the other groups, meaning that the groups were more discriminable than either of the randomly assigned groups. In all cases the rank order of significance was TvT <AvA<AvT. A somewhat surprising result of this analysis is that the significance value of AvA, while generally not low enough to be truly significant, is always lower than the value for TvT. It is possible, but unlikely that this is a function of chance ($P<1/2^6=0.016$). More likely, it shows a difference in variability of calls in alone situations vs. together situations.

Bat	Alone vs. Alone	Together vs. Together	Alone vs. Together
Brent	0.026*	0.637	0.003*
Dedalus	0.294	0.404	0.058
Farah	0.144	0.800	0.120
Lenny	0.281	0.663	0.014*
Rose	0.749	0.952	0.004*
Winston	0.769	0.968	0.123

Table 3: Significance values for discriminate function analysis of randomly generated selections of calls within each category. * denotes significance $P \leq 0.05$

I also performed t-tests based on individual variables to determine whether they were affected or modified by the bat when it was alone compared to when it was with another bat. The t-test gave individual significance levels, which I recorded for each variable and each bat from a two tailed t-test, using the results when equal variances were not assumed. This helped to determine which individual statistics were most meaningful for an individual bat. Table 4 shows

Bat	Cdur†	St freq h1	Mid freq h1	End freq h1	Bandwidth	T50	Curvature	H1 max amp	H1 max freq	Lp MSE
Brent	0.028*	0.000*	0.001*	0.152	0.000*	0.000*	0.476	0.000*	0.065	0.039*
Dedalus	0.094	0.072	0.000*	0.000*	0.375	0.038*	0.494	0.000*	0.053*	0.002*
Farah	0.505	0.898	0.223	0.053*	0.460	0.821	0.245	0.000*	0.643	0.060
Lenny	0.683	0.475	0.042*	0.000*	0.117	0.027*	0.001*	0.000*	0.834	0.000*
Rose	0.007*	0.572	0.517	0.056	0.010*	0.009*	0.955	0.004*	0.669	0.145
Winston	0.786	0.600	0.724	0.332	0.969	0.884	0.158	0.000*	0.591	0.004*

Table 4: Significance values of two-tailed t-tests of a selection of variables. Equal variances were not assumed for these calculations. Significant values mean that a change was in the same direction with each other bat. Changes in different directions with different bats will not produce significant results. * denotes significance P≤0.05. A list of variable abbreviations is provided at the end of the paper.

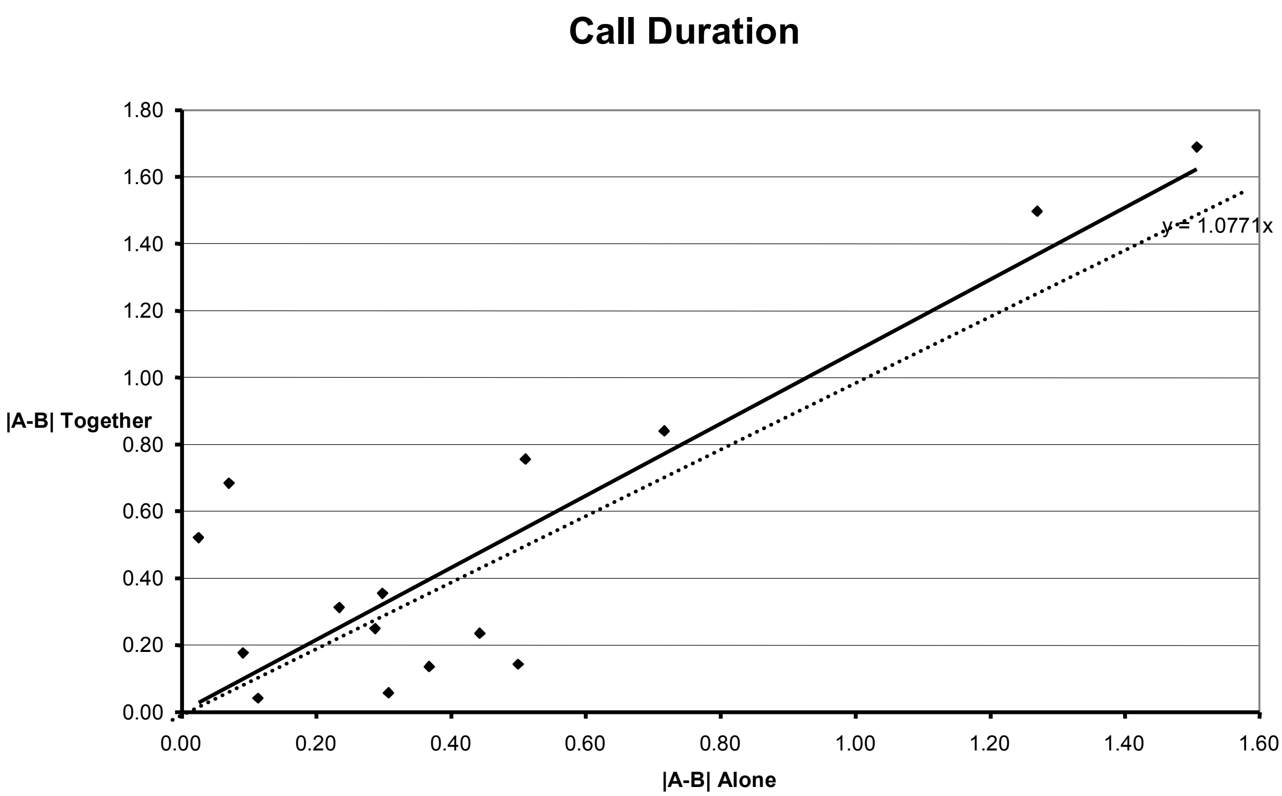
a few of the t-test results, including those which were easy to comprehend, and all variables in which four or more of the bats had significant t-test results between alone and together.

Variable	Slope
Cdur	1.077±0.116
St freq h1	0.987±0.167
Mid freq h1	1.084±0.237
End freq h1	1.009±0.111
Bandwidth	0.842±0.079*
T50	0.885±0.144
Norm T50	0.793±0.067*
Curvature	0.867±0.055*
H1 max amp	1.028±0.186
H1 max freq	0.971±0.178
Lp MSE	1.083±0.157

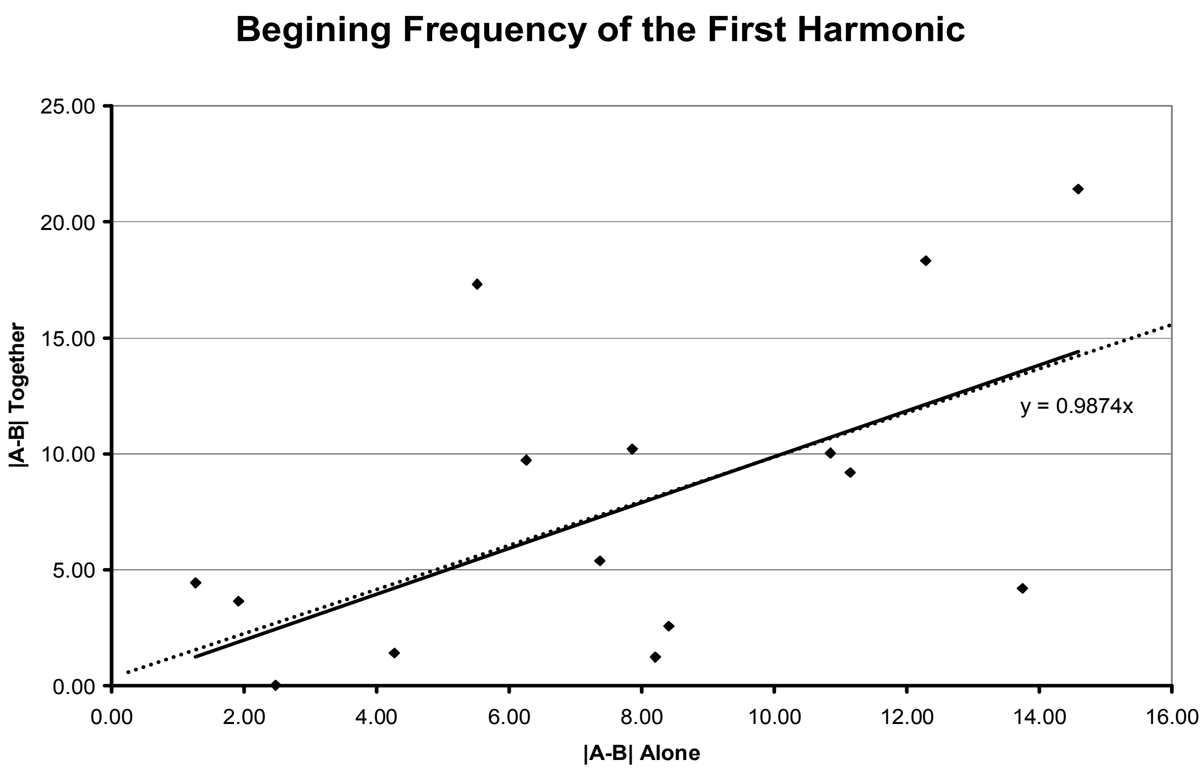
Table 5: Slopes for graphs of each variable. * denotes significant difference from 1. Slopes of greater than 1 mean that calls tend to increase in difference while slopes of less than one mean that calls tend to be more alike.

Maximum amplitude is notable for its very high significance for all bats. The mean value for maximum amplitude when up in each case, meaning bat calls are louder when they are flying with another bat than when they are flying alone.

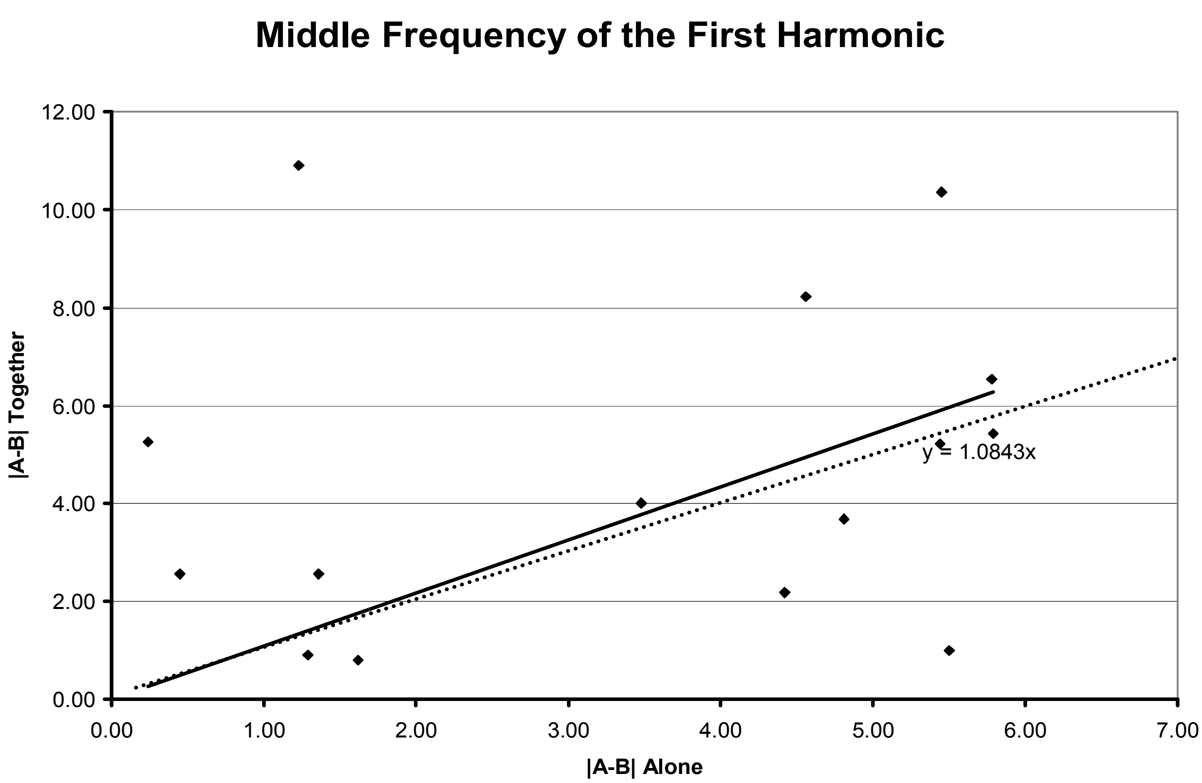
The above variables, plus the full bandwidth (starting frequency – ending frequency) and a normalized t50 (t50÷call duration), were used in a final analysis of trends in the observations. I wanted to calculate whether bats always changed their calls in the same way when they were flying with another bat, regardless of which bat that was. To do this, I divided each bat's calls into groups by which day it was (i.e. which bat he or she was with) and by whether they were alone or with another bat. SPSS calculated the average value of each of the variables by group. I used these to create a scatter plot of each variable so that each point was a pair of bats, with their alone calls on the Xaxis and their together calls on the Y-axis (Graphs 1-11; dashed line represents a slope of 1). Values for X and Y were calculated by subtracting the absolute value of bat A's average from bat B's average. (|A-B|). No change would produce a regression line through the origin with a slope of 1, so I was looking for variables that had a different slope. SPSS was used to calculate both the slope and the mean squared error (MSE) of the slope for each variable (SPSS Inc., 2004). The bats did not seem to have increased the distance between their own calls and those of other bats in this experiment as shown by Table 5.



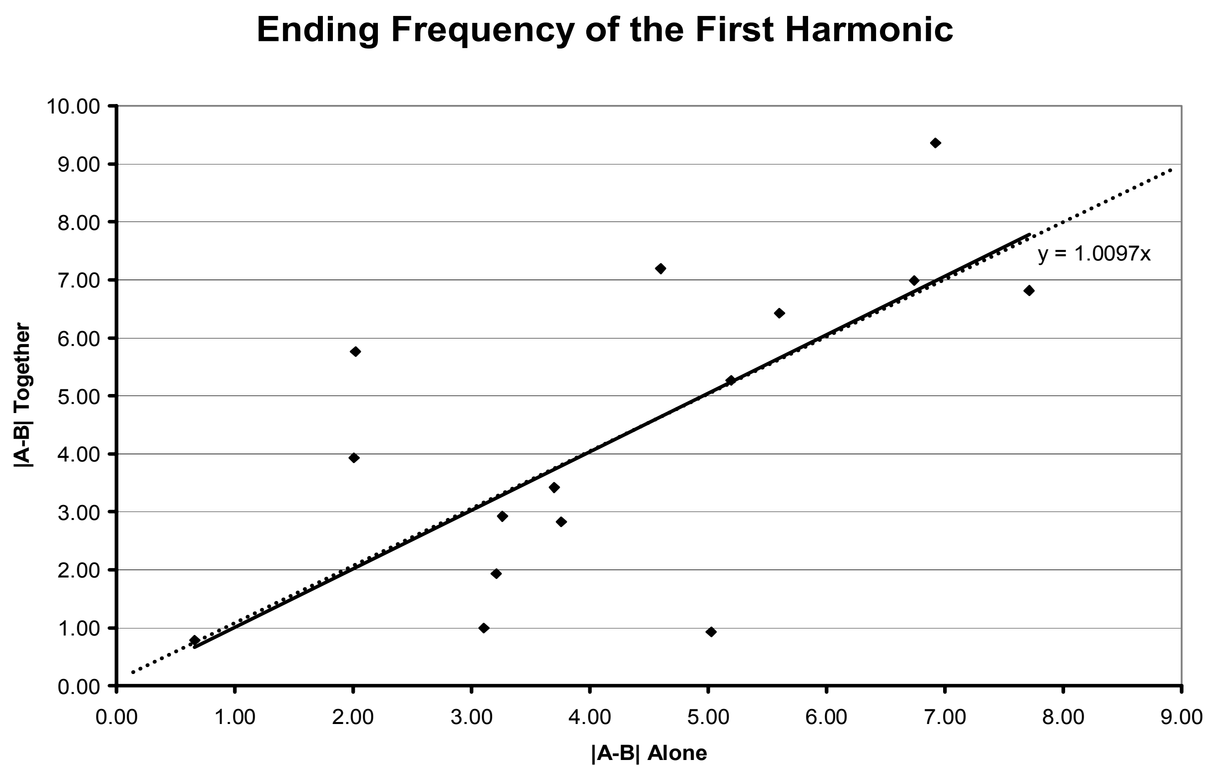
Graph 1: $R^2 = 0.704$



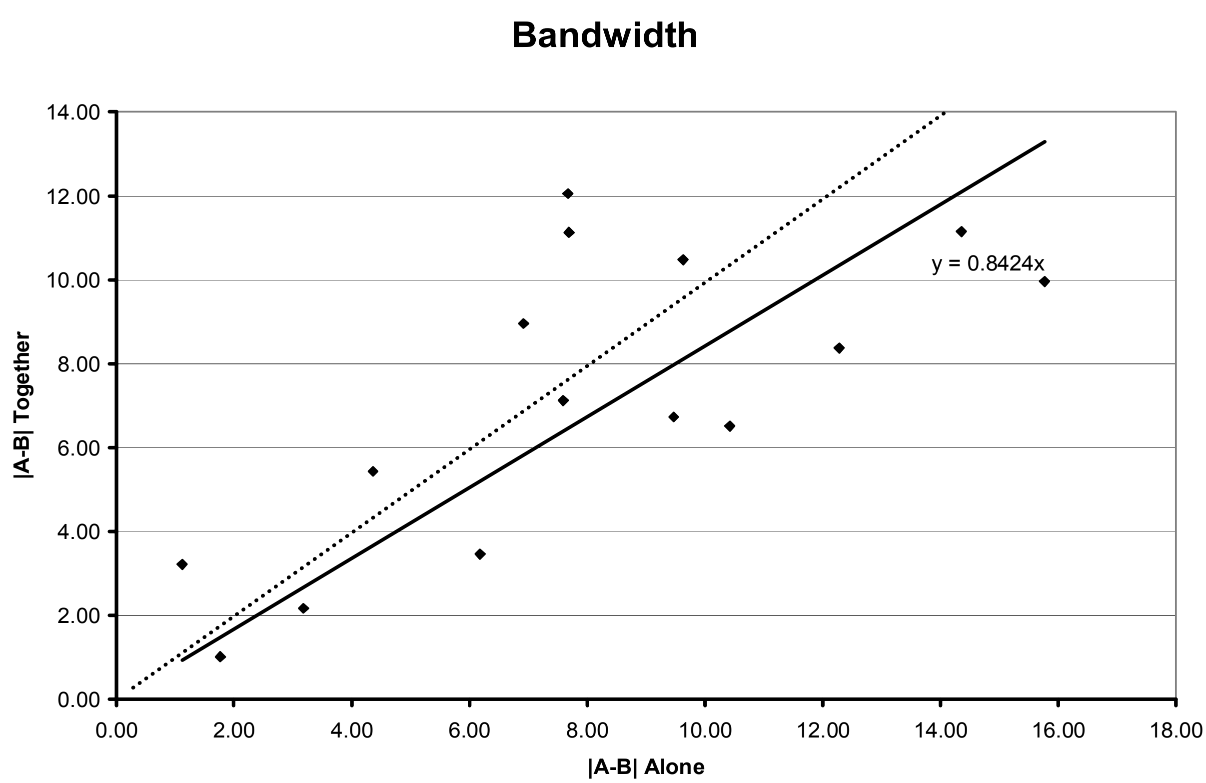
Graph 2: $R^2 = 0.278$



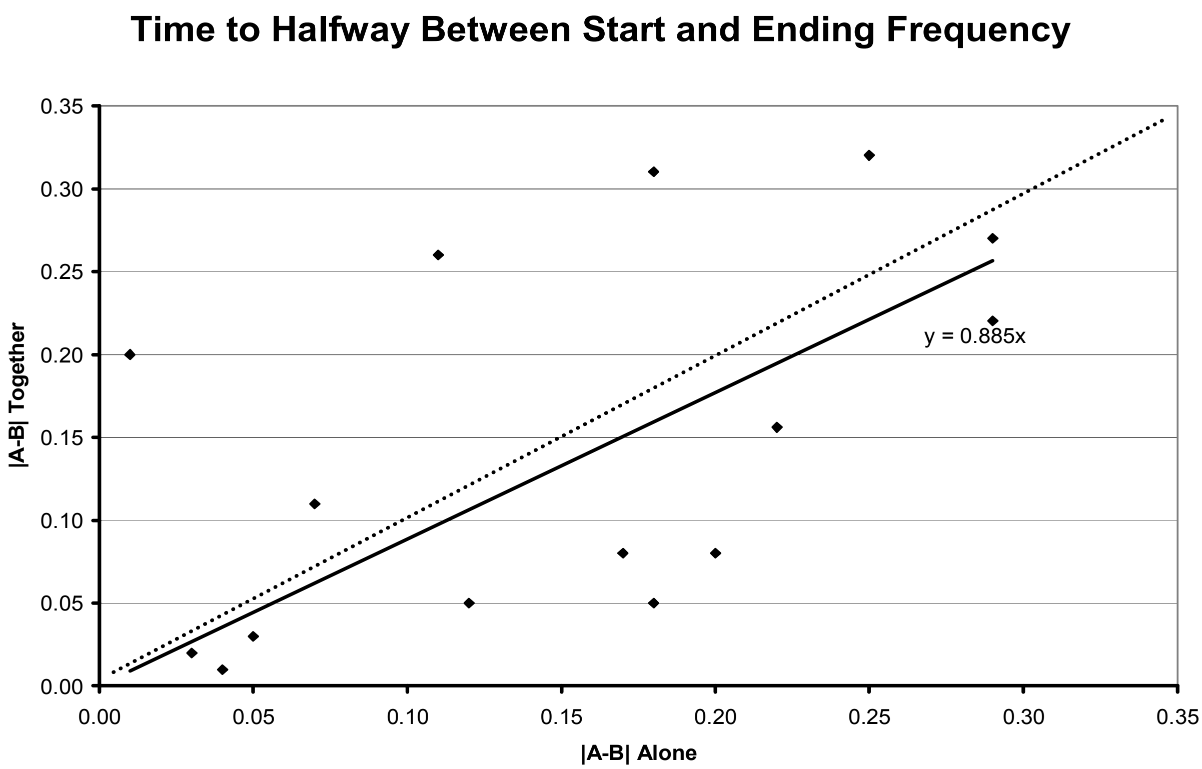
Graph 3: $R^2 = -0.279$



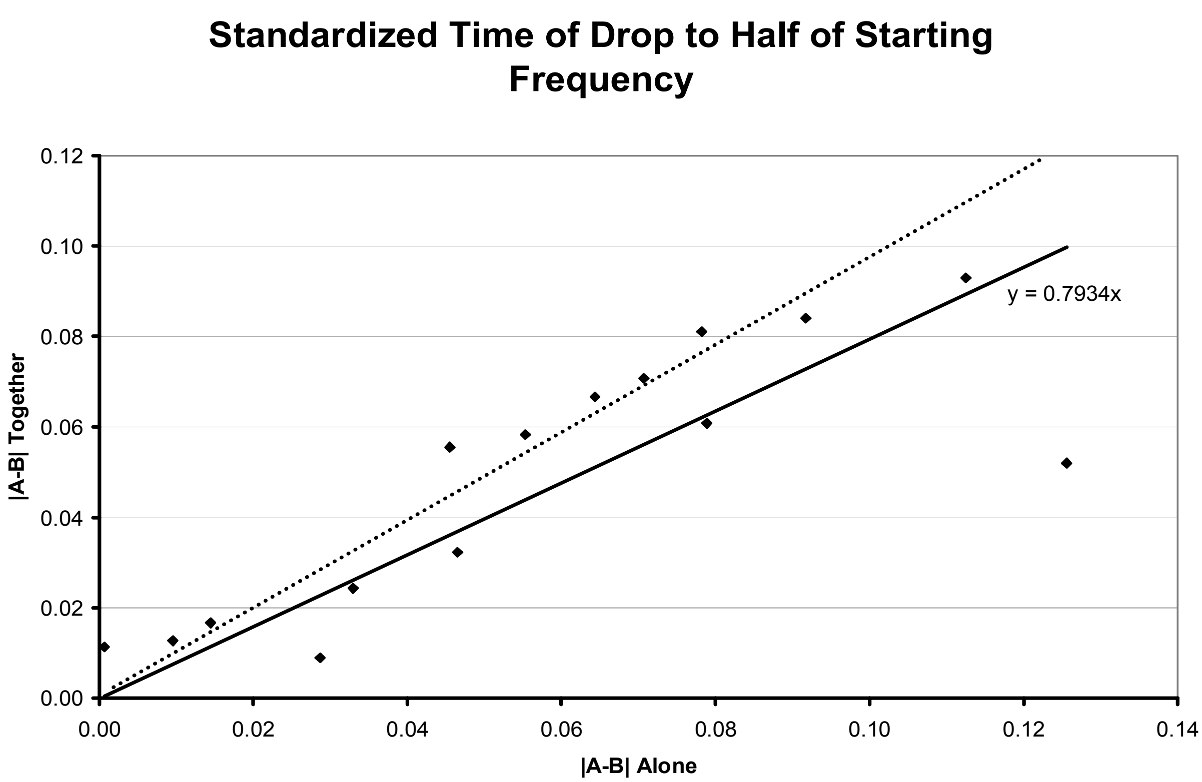
Graph 4: $R^2 = 0.443$



Graph 5: $R^2 = 0.409$

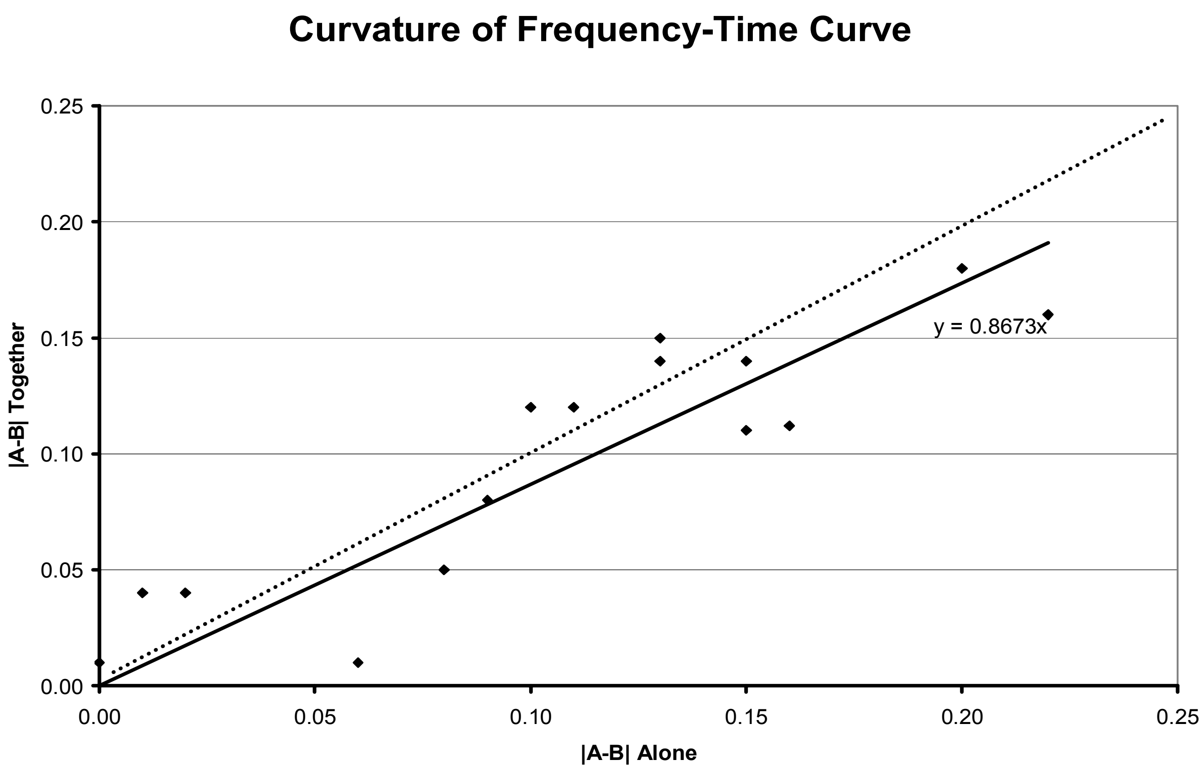


Graph 6: $R^2 = 0.230$

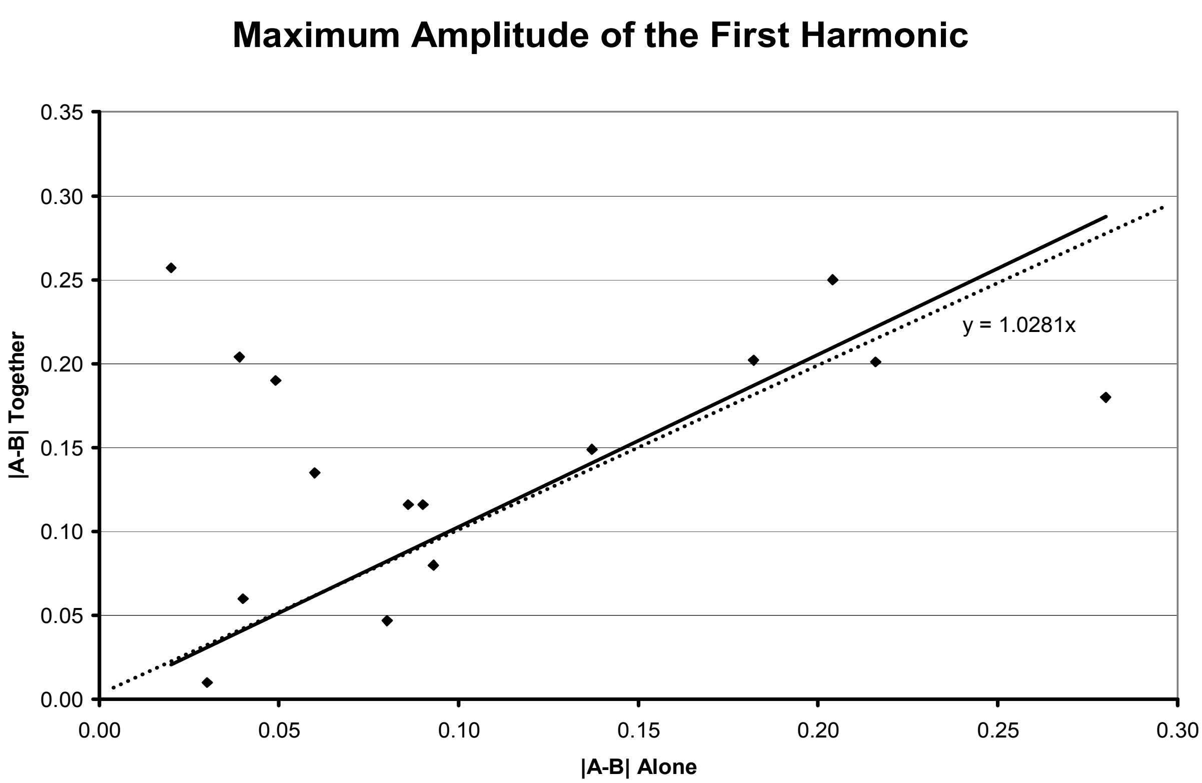


Graph 7: $R^2 = 0.634$

Curvature of Frequency-Time Curve

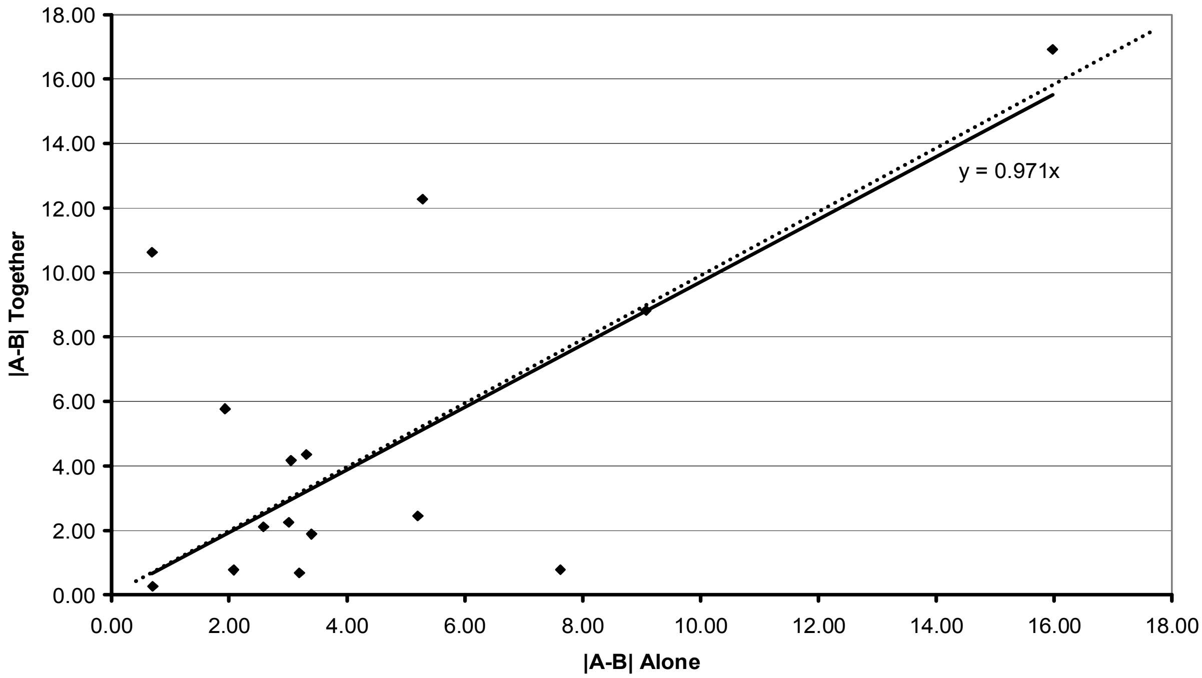


Graph 8: $R^2 = 0.772$



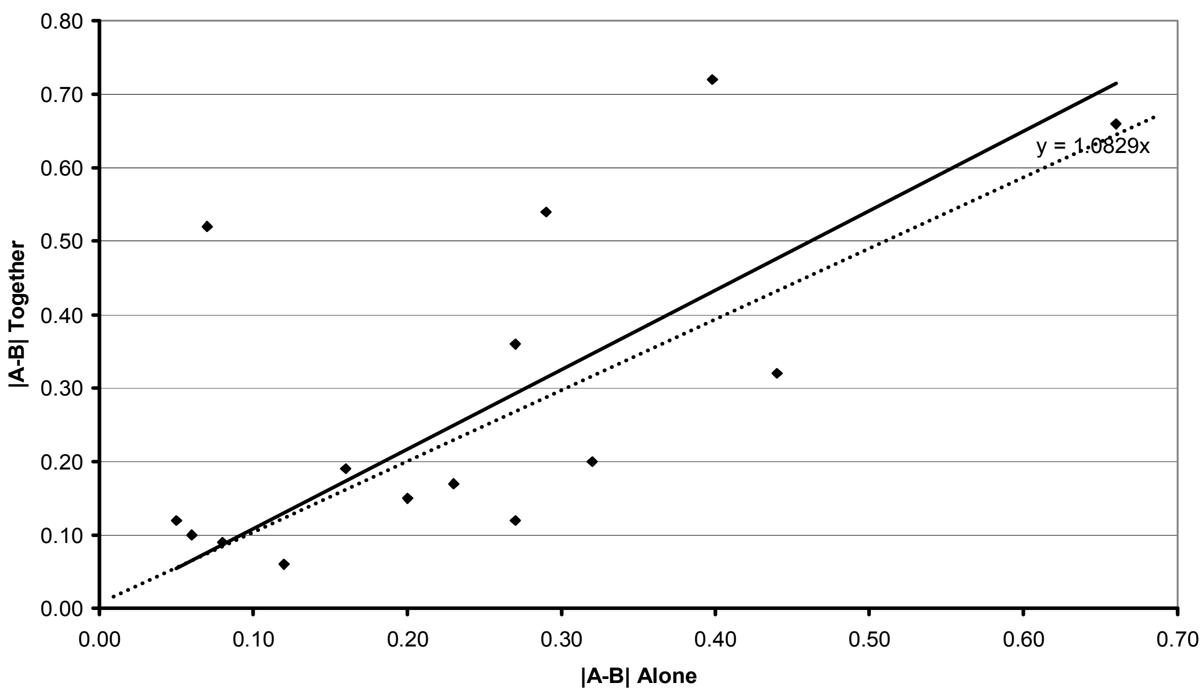
Graph 9: $R^2 = -0.611$

Maximum Frequency of the First Harmonic



Graph 10: $R^2 = 0.344$

Linear Period Mean Squared Error



Graph 11: $R^2 = 0.360$

Discussion

The results of the trend analysis were surprising in many ways. The DFA showed a difference between calls emitted alone and calls emitted with other bats, but little else corresponded with that difference. It is possible that the principle components we used from the PCA relied on maximum amplitude and that was a major factor in group discrimination. I expected to find several variables with a slope greater than 1, which would suggest a jamming avoidance response (Ulanovsky, Fenton, Tsoar, and Korine, 2004). Jamming of signals occurs when a bat tries to receive and process a signal which it did not send, and gets confused by the information contained, or when it tries to process the echo from one call as being the echo from another. There are many possibilities for this result. One possibility is that while the bats do change their calls, they do so in a random, non-directional manner. This seems counterintuitive, but it may work if bats first assess the calls of each other and then alter their calls to make them more different based on the individual they are flying with. This does not seem to have been the case in my experiment. We were able to divide them into groups of alone and together quite easily using DFA. Another possibility is that our bat's calls were so well known to each other from living near each other that they did not need to change very much between alone and together situations. They may have already developed distinct calls from one another, or they may be able to recognize the other bat's calls from hearing it so often before. If the call is very individualized or well known jamming would not likely be much of an issue (Masters, Raver, and Kazial, 1995). There is also the possibility that the bats simply do not experience jamming when only one other bat is present, so calls do not change very much because they don't need to.

Call changes in bats are a promising area for a lot more research. Looking at changes correlated with flying with different individuals is one area that has not been well studied. Sex has also been shown to be encoded in echolocation calls (Kazial and Masters, 2004), so studies looking at whether call changes are correlated with an individual's sex, or the sex of the bat that it is flying with would add a great deal more information on this topic. Studies like this with other species of bats are also important in determining whether these conclusions can be generalized for bats or whether they are specific to this species or genus.

†Abbreviations for Call Parameters

Cdur – call duration in milliseconds (ms)

St freq h1 – starting frequency of the first harmonic; 20 db down from the maximum amplitude

Mid freq h1 – middle frequency of the first harmonic

End freq h1 – ending frequency of the first harmonic; 20 db down from the maximum amplitude

Bandwidth –starting frequency minus ending frequency

T50 – time from the beginning of the call to the middle frequency in ms

Norm T50 – time to middle frequency adjusted for call duration, i.e. T50/Cdur

Curvature – a measure of the curve of the frequency x time graph of a call, 0 is a straight line and 1 is a right angle

H1 max amp – maximum amplitude of the first harmonic

H1 max freq – frequency of the first harmonic at H1max amp

Lp MSE – linear period mean squared error

Acknowledgements

I would like to thank Dr. W.M. Masters for his help and advice with all areas of this study, Kelly Hoffman, for helping to fly and record the bats, and to both of them and Joe Wernet for helping with animal care.

References

Barnard, S.M. (1995). *Bats in captivity.* Wild Ones Animal Books: Springville, CA.

Boughman, J.W. and Wilkinson G.S. (1998). Greater spear-nosed bats discriminate group mates by vocalization. *Animal Behaviour.* 55:1717-1732.

Burnett, S.C., Kazial, K.A., and Masters, W.M. (2001). Discriminating individual big brown bat (*Eptesicus Fuscus*) sonar vocalizations in different recording situations. *The International Journal of Animal Sound and its Recording.* 11:189-210.

Engineering Design (2002). NIDisk version 1.30. www.engdes.com

Fenton, M.B. (2003). Eavesdropping on the echolocation and social calls of bats. *Mammal Review.* 33(3):193-204.

Jones, G. (1999). Scaling of echolocation call parameters in bats. *The Journal of Experimental Biology.* 202:3359-3367.

Kazial, K.A., Burnett, S.C., and Masters, W.M. (2001). Individual and group variation in echolocation calls of big brown bats, *Eptesicus Fuscus* (Chiroptera: Vespertilionidae). *Journal of Mammalogy.* 82(2):339-351.

Kazial, K.A. and Masters, W.M. (2004). Female big brown bats, *Eptesicus fuscus,* recognize sex from a caller's echolocation signals. *Animal Behaviour.* 67:855-863.

Lachenbruch, P.A. (1968). On expected probabilities of misclassification in discriminant analysis, necessary sample size, and a relation with the multiple correlation coefficient. *Biometrics.* 24(4):823-834.

Leippert, D., Frank, E., Gabriel, P., Kutter, S., Scheidemann, K.D., von Stillfried, N. and Weller, F. (2002). Prey-correlated spectral changes in echolocation sounds of the Indian false vampire *Megaderma lyra. Ethology.* 108:139-156.

Obrist, M.K. (1995). Flexible bat echolocation: The influence of individual, habitat, and conspecifics on sonar signal design. *Behavioral Ecology and Sociobiology.* 36:207-219.

Masters, W.M., Jacobs S.C., and Simmons, J. A. (1991). The structure of echolocation sounds used by the big brown bat *Eptesicus fuscus*: Some consequences for echo processing. *Journal of Acoustical Society of America.* 89(3):1402-1413.

Masters, W.M., Raver, K.A.S., and Kazial, K.A. (1995). Sonar signals of big brown bats, *Eptesicus fuscus*, contain information about individual identity, age, and family affiliation. *Animal Behaviour.* 50:1243-1260.

MathWorks, The (2005). MATLAB version 7.0.4.365 (R14) Service Pack 2. www.mathworks.com

Ratcliffe J.M., ter Hofstede, H.M., Avila-Flores R., Fenton M.B., McCracken G.F., Biscardi S., Blasko J., Gillam E., Orprecio J., and Spanjer G. (2004). Conspecifics influence call design in the Brazilian free-tailed bat, *Tadarida brasiliensis*. *Canadian Journal of Zoology.* 82:966-971.

SPSS Inc. (2004). SPSS 13.0 for Windows.

Surlykke A., and Moss, C.F. (2001). Echolocation behavior of big brown bats, *Eptesicus fuscus,* in the field and the laboratory. *Journal of the Acoustical Society of America.* 108(5) part 1:2419-2429.

Ulanovsky, N., Fenton, M.B., Tsoar, A., Korine, C. (2004). Dynamics of jamming avoidance in echolocating bats. *Proceedings: Biological Sciences.* 271(1547):1467-1475.